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# Appearance of a Nuclear Histone H1 Kinase at the Start of DNA Synthesis of Regenerating Rat Liver

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**ABSTRACT**—Phosphorylation of histone H1 and elevation of nuclear protein kinase activity occur at the start of DNA synthesis during regeneration of rat liver. X ray irradiation prior to partial hepatectomy depressed both elevation of the nuclear protein kinase activity and DNA synthesis. Thus, the protein kinase seemed to play a role in the start of DNA synthesis, but the property of the enzyme was not well characterized. The nuclear protein kinase activity was measured with casein or histone H1 as substrate. A protein kinase activity specific for histone H1 was found in the extract from the regenerating liver nuclei, but not that from the irradiated and partially hepatectomized liver nuclei. On the other hand, casein kinase activity was found in the extract from the regenerating liver irrespoctive of prior X ray irradiation. Gel filtration chromatography of the nuclear extract revealed the presence of the histone H1 kinase and two casein kinases in the regenerating liver. Histone H1 kinase was observed in the regenerating liver, but not in the non-hepatectomized nor the irradiated and hepatectomized liver. Two casein kinases were found in the non-hepatectomized liver and partial hepatectomy with or without prior X irradiation induced a little increase in the activites. The present results indicate that appearance of the nuclear histone H1 kinase activity is required for phosphorylation of histone H1 and the start of DNA synthesis in the regenerating liver.

## INTRODUCTION

Hepatocyte in the adult liver does not proliferate in a normal physiological state. But when a part of the liver is removed, the rest of the liver starts proliferating and when the liver mass has gained the former size the cells return to the quiescent state. Proliferation of hepatocytes are stimulated by several growth factors, such as hepatocyte growth factor or hepatopoietin A [1]. Before the onset of DNA synthesis the stimulated cells show many responses such as increase in $Na^+$- $H^+$ exchange, polyamine metabolism, and amino acid transport [2]. Phosphorylation of histones and HMG proteins which belong to nuclear non-histone proteins, and synthesis of histones and HMG (high mobility group) proteins also occur before and during DNA synthesis, and the enzymes of nucleotide motabolism are synthesized at the same period [2]. However, causal relatiopnships among these events are not clear. A dose of X rays enough to inhibit the onset of DNA synthesis inhibited phosphorylation and synthesis of histone H1, while it failed to inhibit phosphorylation and synthesis of HMG proteins [2, 3]. Thus, phosphorylation of histone H1 seemed to be closely related to the onset of DNA synthesis. Changes in nuclear protein kinase activity were measured, therefore, during the prereplicative phase of regeneration in the liver of partially irradiated rat [4]. The protein kinase activity increased at the onset of DNA synthesis and the rise of the activity was inhibited by X ray irradiation delivered just before partial hepatectomy [4]. Consequently this protein kinase seemed to be responsible for phosphorylation of histone H1, although its nature was unknown.

Rat liver nuclei contain several kinds of protein kinases such as histone H1 kinase [5, 6], casein kinases [7, 8], protein kinase C [9], Ca/calmodulin-dependent protein kinase [10] and cAMP-dependent protein kinase [4, 11]. Our previous

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results suggested that growth-associated histone kinase of Langan, a protein kinase specific for histone H1 found in growing cells [5], was responsible for the rise of the protein kinase activity in the nuclei, although participation of other kinase could not be excluded completely [4]. Therefore, we measured histone H1 kinase and casein kinase activities in parallel. The present results indicate that nuclear kinase specific for histone H1 is responsible for the rise of protein kinase activity at the onset of DNA synthesis.

## MATERIALS AND METHODS

### *Animals and materials*

Male Wistar strain rats, supplied from our breeding colony, were used between 8 and 10 week old of age. [$\gamma$-$^{32}$P]ATP was purchased from Amersham, Japan. PMSF (phenylmethylsulfonyl fluoride), $\alpha$-casein (Sigma), and Combithek (protein standard set: Boeringer) were used. Histone H1 was extracted from rat liver according to the procedures of Sanders and Johns [12], and the acid soluble fraction was passed through a column of BioRex 70 (Bio-Rad Lab., USA) [13]. The fraction containing histone H1 was collected, freeze-dried, and dissolved in water. Fractions containing mainly histone $H1_0$ were not used. Histone H1 solution, which showed a single band with polyacrylamide gel electrophoresis, was divided in small aliquots and stored at $-20^{\circ}C$ until use.

### *X ray irradiation and partial hepatectomy*

X ray irradiation and partial hepatectomy of rats were carried out as described previously [3, 4]. During anesthesia with pentobarbital, rats were irradiated to their upper abdomen including the liver with 4.8 Gy of X rays. The condition of irradiation was 200 kVp and 20 mA and X rays were filtered through 0.5 mm Cu and 0.5 mm Al. Following irradiation, the median and the left lateral lobe of the liver were removed. The non-irradiated rats were also hepatectomized under anesthesia with pentobarbital. The right lateral and caudate lobes were used in the experiments of non-hepatectomized rats, since these lobes were used in the case of hepatectomized rats. The hepatectomized rats were killed at 24 h after partial hepatectomy, when DNA synthesis started [3] and the protein kinase activity increased [4].

### *Preparation of nuclear extract*

Isolation of nuclei, essentially according to the procedure of Blobel and Potter, and preparation of the nuclear extract were described previously [4]. Briefly, the nuclei were extracted with 0.4 M NaCl in buffer A, which consisted of 20 mM TrisHCl pH 7.5, 1 mM EDTA pH 7.5, 0.1 mM PMSF, 5 mM benzamidine, 2 mM glycerol-2-phosphate and 10 mM 2-mercaptothanol. After treatment with BioRex 70, the extract was mixed with potassium phosphate pH 7.5 and cAMP to give 0.1 M and 1 $\mu$M respectively in order to separate cAMP-dependent protein kinase [5, 6]. The protein precipitated with ammonium sulfate between 17.5% and 35% saturation was collected. The precipitate from one rat (ca. 5 g liver) was dissolved in 0.5 ml buffer A containing 0.4 M NaCl. The extracts thus obtained were dialyzed overnight against about 50 volume of buffer A containing 10% glycerol. For resolution with chromatography the nuclei from 3 rats were combined before extraction and the extracts were used for gel filtration chromatography immediately after extraction. Although 0.4 M NaCl did not extract all the kinase activity, the portion of the extracted activity was not changed much during regeneration [4]. The protein kinase activities were hence compared on the nuclear extracts with 0.4 M NaCl in the present experiments.

### *Column chromatography of the extract*

FPLC system (Pharmacia) was used. When Mono Q column was used to separate the extract, dialysis of the extract before chromatography caused aggregation of the proteins and almost all proteins remained on the top of the Mono Q column. Therefore, the extract was applied to the column of Superose 12 immediately after extraction. The column was equilibrated in 0.4 M NaCl containing buffer A, and ca. 2 mg of the nuclear extracts were applied. Fractions of 1 ml were collected, and the enzyme activity and protein concentration were determined. Protein was determined according to the procedure of Bensadoun

and Weinstein [14].

*Assay of protein kinase activity*

A similar procedure with the previous experiments was used with rat liver histone H1 and α-casein as substrate [4]. Briefly, the reaction mixture contained in 0.05 ml; 50 mM TrisHCl pH 7.5, 10 mM $MgCl_2$, histone H1 (0.5 mg/ml) or α-casein (0.5 mg/ml), 0.5 mM ATP (containing 1 μCi [$\gamma$-$^{32}$P]ATP), 1 mM EDTA pH 7.5, 1 mM dithiothreitol, and 20 mM glycerol-2-phosphate. The reaction was started with addition of the enzyme. After incubation for 10 min at 37°C, the reaction was stopped with addition of 0.25 ml 20% trichloroacetic acid. The precipitate was collected on a Millipore filter and washed with 10% trichloroacetic acid. After drying in air, radioactivity of the filter was measured with a liquid scintillation counter. Since incubation without substante caused substantial uptake of radioactivity, net uptake of radioactivity was calculated by subtracting the values obtained without substrate. The zero time value was subtracted.

## RESULTS

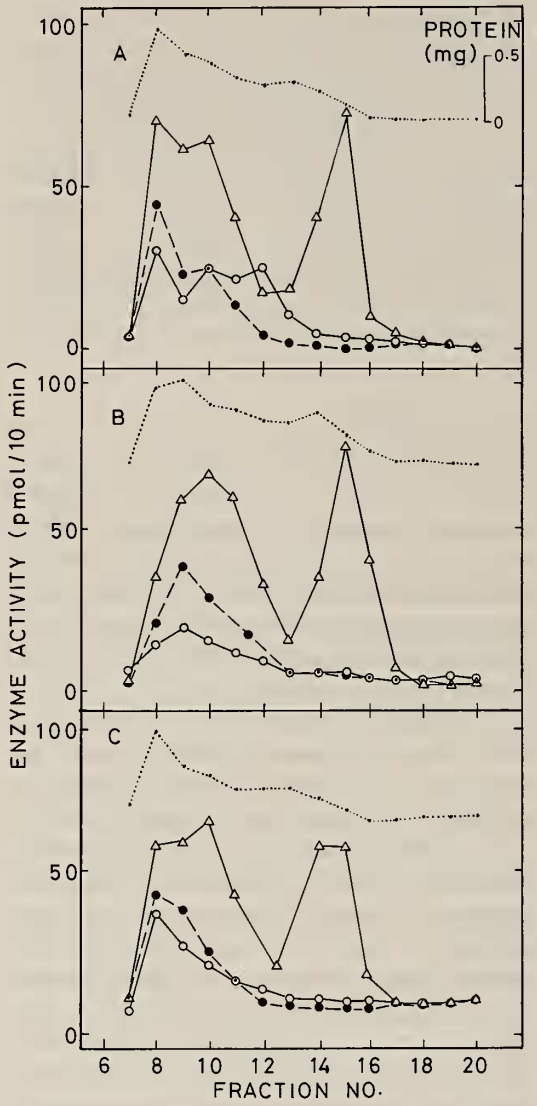
Normal liver nuclei contain a protein kinase or kinases and partial hepatectomy causes partial increase in kinase activity at the time of DNA synthesis [4]. More than one protein kinase could be measured simultaneously in the previous experiments, however, since histone mixture used as the substrate contained histone mixture as well as non-histone proteins (data not shown). In the present experiments, therefore, protein kinase activity was measured with more defined substrate; rat liver histone H1 and casein. Phosphorylation due to endogenous substrate was subtracted from the values with the substrates, and also shown in the results separately (Table 1). The protein kinase activity phosphorylating histone H1 was observed in the extract from regenerating liver nuclei, while in was not observed in the extract from the nuclei of the X ray-irradiated and partially hepatectomized rat (Table 1). On the other hand, the protein kinase activity phosphorylating casein was observed in both the hepatectomized and the irradiated and hepatectomized nuclear extract. Phosphorylation of endogenous substrate was also observed on all the groups measured (Table 1). Irradiation caused no substantial changes in the activity phosphorylating casein or endogenous substrate. These results indicated presence of histone H1 kinase distinct from casein kinase 1 and 2 and responsible for phosphorylation of histone H1 at the onset of DNA synthesis in the regenerating liver nuclei.

Table 1. Protein Kinases in the Nuclear Extract of Regenerating Liver

| Substrates | Activity (nmol/10 min/mg protein) | | |
|---|---|---|---|
| | Non-irradiated | X-irradiated | |
| histone H1 | 3.66±0.93 | 0.63±0.57 | $P<0.05$ |
| casein | 4.75±1.60 | 3.65±0.80 | NS |
| (endogenous) | 7.14±1.66 | 5.71±1.57 | NS |

The nuclear extract was prepared from the rat at 24 h after partial hepatectomy. Mean of 3 independent experiments with SEM.

To confirm the presence of a protein kinase more specific to histone H1 than casein in the nuclei, the nuclear extracts were fractionated with gel filtration chromatography using Superose 12 column. The nuclear extract dissolved in buffer A containing 0.4 M NaCl was immediately applied to Superose 12 column equilibrated in the same medium. The protein kinase activities of each fraction was measured in the presence of histone H1 or casein or without substrate addition (Fig. 1). Elution profile of proteins did not change much among the non-hepatectomized, the hepatectomized and the X-irradiated and hepatectomized liver. The activity phosphorylating endogenous substrate(s) was found in the fraction eluted at the void volume and decreased in the later fractions. Endogenous substrate(s) were probably associated with its protein kinase in the nuclear extract and pass through the column in the associated form. The activity phosphorylating casein was eluted in two peaks, i.e. fraction 10 and 15 as maximal respectively, while that phosphorylating histone H1 was maximal in fraction 12. The liver nuclei have been reported to contain two casein kinases [7, 8]. The two peaks probably correspond to the nuclear casein kinase 1 (fraction 15) and 2 (fraction




10) or NI and NII [7, 8]. The casein kinase activity after subtraction of the activity without substrate addition were divided into two parts, i.e. the fractions 13–17 and the fractions 7–12, and they were designated here as casein kinase 1 and 2 respectively. The integrated activities are shown in Table 2. Activities of casein kinases of hepatectomized liver are a little higher than those of non-hepatectomized liver, but X ray irradiation hardly affected the activities.

An activity phosphorylating histone H1 was eluted in the fractions of regenerating liver between casein kinase 2 and 1. This indicates that casein kinases and histone H1 kinase are different enzymes. The nuclear extract from the non-hepatectomized liver or from the X-irradiated and hepatectomized liver contained casein kinase 1 and 2, but no histone H1 kinase. The activity phophorylating endogenous substrate was subtracted from that in the presence of histone H1 for each fraction. Phosphorylating activities of fractions 8 to 9 or to 11 were lower in the presence of histone H1 than in its absence, indicating that histone H1 inhibited the phosphorylation of the endogenous substrate. The fractions, where the activity was higher in the preence of histone H1

FIG. 1. Gel filtration chromatography of the nuclear extracts from rat liver. Superose 12 attached to FPLC system was used. (A) Partially hepatectomized (2.54 mg protein), (B) partially irradiated and hepatectomized (2.58 mg protein), and (C) non-hepatectomized (2.58 mg protein). Histone H1 ( ), casein (△) and none (●) were used as substrate. Activity in 25 μl of the eluate was shown in the figure. ...., Protein contents.

TABLE 2. Protein Kinases in the Nuclear Extract of Rat Liver

| Rat | X rays | No. of Exp. | Activities (nmol/10 min/mg protein) | | |
|---|---|---|---|---|---|
| | | | HK | CKI | CKII |
| Non-HPX | – | 3 | 0.58±0.09 | 10.87±0.78 | 3.88±0.54 |
| HPX | – | 2 | 2.31±0.46* | 17.52±4.78 | 4.74±1.42 |
| HPX | + | 3 | 0.62±0.36 | 15.30±1.38* | 4.16±1.33 |

The nuclear extract from the non-hepatectomized (non-HPX) or the hepatectomized (HPX) rat was used for FPLC. Protein kinase activities and protein contents of each fraction were measured and summed up. HK; histone H1 kinase, CKI and CKII; casein kinase 1 and 2 respectively. The asterisks indicate a significant difference from the non-hepatectomized ($P<0.05$).

than in its absence, were regarded to contain histone H1 kinase and their differences are summed up (Table 2). Nuclear histone H1 kinase activity of the hepatectomized liver was higher than either the non-hepatectomized or the X ray-irradiated and hepatectomized liver. Partial inactivation of the histone H1 kinase however was noticed on the fractions after chromatography, since the specific activity after chromatography was similar to that before chromatography (compare Table 2 with Table 1). The reason of inactivation is unknown, but instability of growth associated histone kinase after chromatography was reported [6, 15].

Apparent molecular weight of three protein kinases were calculated from chromatography of the standard proteins; ferritin, catalase, aldolase, bovine serum albumin, egg albumin, chymotrypsinogen A and cytochrome *c*. Some of the standard proteins, especially ferritin, showed peakds with smaller molecular weight after chromatography. This would be due to depolymerization of the polymerized form of the proteins in the elution buffer containing 2-mercaptoethanol. These peaks with smaller molecular weight were also used for calculation. The obtained regression line was log(molecular weight)$=5.32-0.137\times$(retention time in min); $r=-0.9646$. The calculated sizes of the protein kinases were 381 kDa (casein kinase 2), 92 kDa (histone H1 kinase), and 12 kDa (casein kinase 1), respectively (data not shown).

The results shown in Figure 1 indicated that histone H1 inhibited phosphorylation of endogenous substrate by the kinase eluted simultaneously. Phosphorylation of endogenous substrate and casein was then measured in the presence of different concentrations of histone H1, since casein kinases were the protein kinase found in the nuclei in the present condition except for histone H1 kinase (Table 3). Histone H1 inhibited phosporylation of casein by casein kinase 1 (fraction 15) or 2 (fraction 10). Phosphorylating activity of fraction 8 which contained endogenous substrate was activated with casein and the activity was inhibited by histone H1 irrespective of addition of casein.

Table 3. Inhibition by histone H1 of the phosphorylating activity of the nuclear extract with or without casein

| Enzyme | Casein | histone H1 (mg/ml reaction mixture) | | |
|---|---|---|---|---|
| | | 0 | 0.5 | 1.0 |
| | | nmol/10 min/mg protein | | |
| Fr. 8 | + | 5.50 | 6.56 | 3.32 |
| | − | 2.54 | 1.62 | 1.18 |
| Fr. 10 | + | 10.98 | 9.41 | 5.04 |
| | − | 1.63 | 1.91 | 1.33 |
| Fr. 15 | + | 37.17 | 17.32 | 16.73 |
| | − | 0.80 | 1.33 | 1.98 |

The values with casein are not corrected for $^{32}P$ uptake without casein. Fraction (Fr.) 10 and Fr. 15 contain casein kinase 2 and casein kinase 1 respectively.

## DISCUSSION

Phosphorylation of histone H1 is closely related to the initiation of DNA synthesis of regenerating liver [2, 3]. However, it is still not known which enzyme is responsible for the phosphorylation. Although cAMP-dependent protein kinase was found in the nuclei [11], the enzyme was not related with phosphorylation of histone H1 at the time of DNA synthesis [4] and was removed from the nuclear extract in the present experiments through treatment with cAMP and ammonium sulfate precipitation [5, 6]. Protein kinase C and $Ca^{2+}$/calmodulin-dependent protein kinase II are also reported in rat liver nuclei [9, 10], but the present assay condition was not favorable for the Ca-dependent kinases, since the assay medium contained no $Ca^{2+}$ but EDTA. The inhibitors of the protein kinase C did not inhibit the nuclear kinase [4]. These protein kinases, therefore, will not be considered further in this article.

The present results show that the nuclear extract of the regenerating liver contained two kinds of casein kinases and one histone H1 kinase. The extract seemed to contain another protein kinase which was associated with endogenous substrate. This will be a casein kinase as discussed later.

Two kinds of casein 1 (NI) and 2 (NII), although the molecular weight of casein kinases in the present results are not consistent with the reported

values (e.g., 50 and 133 kDa [8] or 35 and 200 kDa [15] respectively). In the present paper however they are called casein kinase 1 and 2. Activities of casein kinases increased a little in the regenerating liver and X rays enough to inhibit phosphorylation of histone H1 scarcely affected the increase. A nuclear non-histone protein, HMG 14 is phosphorylated with casein kinase 2 [8, 16] and X rays do not affect phosphorylation of HMG 14 [3]. These are in consistent with the results that X rays scarcely affected the activity of casein kinase.

Both casein kinase 1 and 2 are inhibited by histone H1. An endogenous inhibitor protein of casein kinase was reported previously, which was a 25 kDa protein found in high mobility group (HMG) protein fraction of rat liver, but it was not characterized further [17]. However, histone H1 was not removed from their HMG fraction, which usually contained histone H1, before separation of the inhibitor [17]. The present results suggest that this endogenous inhibitor protein of casein kinase is histone H1. Inhibition by histone H1 of nuclear casein kinase may have physiological significance for regulating phosphorylation of nuclear proteins, although more experiments should be carried out before concluding histone H1 as a natural inhibitor of nuclear casein kinase.

A protein kinase is associated with endogenous substrate and that is the cause of high phosphorylation rate without added substrate (Table 1). The enzyme was able to phosphorylate casein (or it was activated with casein) and it was inhibited by histone H1. It was reported that casein kinase 2 in the nuclear extract was associated firmly with high molecular weight protein such as nucleolin or progesterone receptor [18, 19]. Hence, the enzyme phosphorylating endogenous substrate may be casein kinase 2.

Histone H1 kinase was found in the regenerating nuclei, while it was not observed in the non-hepatectomized nuclei. X irradiation inhibited the appearence of the histone H1 kinase activity in the regenerating nuclei. Casein kinases had a very low affinity for histone H1 as substrate. Therefore, the nuclear histone H1 kinase will be responsible for phosphorylation of histone H1 at the time of DNA synthesis.

The present results coincide with the previous results that the enzyme responsible for phosphorylation of histone H1 will be a growth associated histone kinase of Langan [4]. Apparent molecular mass of the kinase was 92 kDa. Molecular weight of growth associated histone kinase is not reported, but that of catalytic subunit is 67,000 [6]. Cyclin-$p34^{cdc2}$ protein kinase is specific for histone H1 and is thought to be the same as growth associated histone kinase [20, 21]. Association of $p34^{cdc2}$ with cyclin, 62 kDa, will constitute a complex of 96 kDa, a similar value to the present results, although apparent molecular mass of cyclin-$p34^{cdc2}$ complex is evaluated as 220 kDa from chromatography with Superose 12 [21]. Nuclear localization of the kinase has been shown [22, 23]. Although the cyclin-$p34^{cdc2}$ play a role in the G2 to M transition of the cell cycle, the same enzyme or its homologue is claimed to participate for the G1 to S transition [24–26]. Thus the histone H1 kinase found in the nuclei will be growth associated histone kinase or a kinase related to it, which will play a role in phosphorylation of histone H1 at the onset of DNA synthesis.

The mechanism how X rays inhibit appearance of the histone H1 kinase in the nuclei is not known. It is not known whether X rays affect directly the appearance of histone H1 kinase in the nuclei. Instability of growth-associated histone H1 kinase has been reported [6, 15, 27]. Activity of p34 protein kinase can be controlled by binding of cyclin which is usually synthesized in each cell cycle, and the activity can be changed depending on the phosphorylation state of these two proteins [28, 29]. On the other hand, synthesis of $p34^{cdc2}$ during the G1 phase of stimulated lymphocytes was observed [25]. Hence X rays may suppress appearance of the histone H1 kinase in the nuclei by changing the cellular metabolism resulting in inactivation of the kinase, or by inhibiting *de novo* synthesis of protein(s) constituting the kinase.

## REFERENCES

1 Michalopoulos, G. K. (1990) Liver regeneration: molecular mechanism of growth control. FASEB J. **4**: 176–187.

2 Ord, M. G. and Stocken, L. (1984) Cell and Tissue Regeneration. Wiley, New York.

3 Asami, K. (1987) Synthesis and phosphorylation of histone H1 and high mobility group proteins in the regenerating rat liver after X irradiation. Radiat. Res. **109**: 216–226.

4 Asami, K., Kobayashi, H., Fujiwara, A. and Yasumasu, I. (1989) Changes in the nuclear protein kinase activities in the regenerating liver of partially irradiated rat. Radiat. Res. **119**: 500–510.

5 Langan, T. (1978) Isolation of histone kinases. In "Methods in Cell Biology". Ed. by G. Stein, J. Stein, and L. J. Kleinsmith, Academic Press, New York, Vol. XIX, pp. 143–152.

6 Woodford, T. A. and Pardee, A. B. (1986) Histone H1 kinase in exponential and synchronous populations of chinese hamster fibroblasts. J. Biol. Chem. **261**: 4669–4676.

7 Desjardins, P. R., Lue, R. F., Liew, C. C. and Gornall, A. G. (1972) Purification and properties of rat liver nuclear protein kinases. Canad. J. Biochem. **50**: 1249–1259.

8 Delpech, M., Levy-Favatier, F., Moisand, F. and Kruh, J. (1986) Rat liver nuclear protein kinases NI and NII. Eur. J. Biochem. **160**: 333–341.

9 Masmoudi, A., Labourdette, G., Mersel, M., Huang, F. L. Huang, K-P., Vincendon, G. and Malviya, A. H. (1989) Protein kinase C located in rat liver nuclei. J. Biol. Chem. **264**: 1172–1179.

10 Ohta, Y., Ohba, T. and Miyamoto, E. (1990) $Ca^{2+}$/calmodulin-dependent protein kinase II: Localization in the interphase nucleus and the mitotic apparatus of mammalian cell. Proc. Natl. Acad Sci. **87**: 5341–5345.

11 Laks, M. S., Harrison, J. J., Schwoch, G. and Jungmann, R. A. (1981) Modulation of nuclear protein kinase activity and phosphorylation of histone H1 subspecies during the prereplicative phase of rat liver regeneration. J. Biol. Chem. **256**: 8775–8785.

12 Sanders, C. and Johns, E. W. (1974) A method for the large-scale preparation of two chromosomal proteins. Biochem. Soc. Trans. **2**: 547–550.

13 Seyedin, S. M. and Kistler, W. S. (1979) Levels of chromosomal protein high mobility group 2 parallel the proliferative activity of testis, skeletal muscle, and other organs. J. Biol. Chem. **254**: 11264–11271.

14 Bensadoun, A. and Weinstain, D. (1976) Assay of proteins in the presence of interfering materials. Anal. Biochem. **70**: 241–250.

15 Neuman, J. R., Owens, B. B. and Ingram, V. M. (1979) Nucleosome-associated protein kinases in murine erythroleukemia cells. Arch. Biochem. Biophys. **197**: 447–453.

16 Walton, G. M., Spiess, J. and Gill, G. N. (1985) Phosphorylation of high mobility group protein 14 by casein kinase II. J. Biol. Chem. **260**: 4745–4750.

17 Levy-Favatier, F., Delpech, M., Riffe, A. and Kruh, J. (1985) A 25 000 dalton inhibitor of cAMP independent protein kinases present in rat liver HMG protein preparations. Biochem. Biophys. Res. Communs. **130**: 149–156.

18 Caizergues-Ferrer, M., Belenquer, P., Lapeyre, B., Amalric, F., O'Wallace, M. and Olson, M. O. J. (1987) Phosphorylation of nucleolin by a nucleolar type NII protein kinase. Biochem. **26**: 7876–7883.

19 Logeat, F., Le Cunff, M., Rauch, M., Brailly, S. and Milgron, E. (1987) Characterization of a casein kinase which interacts with the rabbit progesterone receptor. Eur. J. Biochem. **170**: 50–57.

20 Arion, D. and Meijer, L. (1989) M-phase specific protein kinase from mitotic sea urchin eggs: Cyclic activation depends on protein synthesis and phosphorylation but does not require DNA or RNA synthesis. Exp. Cell Res. **183**: 361–375.

21 Brizuela, L., Draetta, G. and Beach, D. (1989) Activation of human CDC2 protein as a histone H1 kinase is associated with complex formation with the p62 subunit. Proc. Natl. Acad. Sci. USA **86**: 4362–4366.

22 Riabawol, K., Draetta, G., Brizuela, L., Vandre, D. and Beach, D. (1989) The cdc2 kinase is a nuclear protein that is essential for mitosis in mammalian cells. Cell **57**: 393–401.

23 Booher, R. N., Alfa, C. E., Hyams, J. S. and Beach, D. (1989) The fission yeast cdc2/suc1 protein kinase: Regulation of catalytic activity and nuclear locarization. Cell **58**: 485–497.

24 Blow, J. J. and Nurse, P. (1990) A cdc2-like protein is involved in the initiation of DNA replication in Xenopus egg extracts. Cell **62**: 855–862.

25 Furukawa, Y., Piwnica-Worms, H., Ernst, T., Kanakura, Y. and Griffin, J. D. (1990) *Cdc2* gene expression at the G1 to S transition in human T lymphocytes. Science **250**: 805–808.

26 D'Urso, G., Marraccino, R. L., Marshark, D. R. and Roberts, J. M. (1990) Cell cycle control of DNA replication by a homologue from human cell of the $p34^{cdc2}$ protein kinase. Science **250**: 786–791.

27 Muller, K. and Little, M. (1989) Chromatin-bound histone 1 kinase activity in synchronized HeLa cells. Exp. Cell Res. **180**: 30–35.

28 Gould, K. L. and Nurse, P. (1989) Tyrosine phosphorylation of the fission yeast $cdc2^{+}$ protein kinase regulates entry into mitosis. Nature, **342**: 39–45.

29 Felix, M.-A., Cohen, P. and Karsenti, E. (1990) Cdc2 H1 kinase is negatively regulated by a type 2A phosphatase in the Xenopus early embryonic cell cycle: evidence from the effects of okadaic acid. EMBO J. **9**: 675–683.